previous problems associated with knowledge-based potentials. These features were obtained for a large set of native and decoy structures and a back-propagating neural network was trained to predict the fitness score. Overall this new scoring potential proved to be superior to the knowledge-based scoring functions used as its inputs. In particular, in the latest CASP (CASP10) experiment our method was ranked third for all targets, and second for freely-modeled hard targets among about 200 groups for the top model predictions. Ours was the only method ranked in the top three for all targets and for hard targets. This shows that initial results from the novel approach are able to capture details that were missed by a broad spectrum of protein structure prediction approaches.

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New Insights on the Mechanism of Action of Ice-Binding Proteins

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Ice-binding proteins (IBPs) aid the survival of cold-adapted organisms by inhibiting the growth of endogenous ice crystals. The binding of IBPs to ice causes a separation between the melting point and the freezing point of the ice crystal (Thermal hysteresis, TH). Although the discovery of IBPs was more than 45 years ago, the mechanism of action is still unclear. For moderately active IBPs it is known that thermal hysteresis values increase with annealing time between ice and IBP before cooling is started. We have extended this observation to hyperactive IBPs. Using a custom-made nanoliter osmometer and a novel microfluidics system [1], we show that the exposure time of crystals to IBPs is a crucial factor and that their activity could be increased up to 40-fold over long periods of time (13 h). We found that each IBP has a different time-dependent behavior thus the structure of the IBP molecule brings about a distinct kinetic behavior. Using our cooled microfluidic system, we show that hyperactive IBPs accumulate progressively on the basal plane, and rapidly on the prism plane. Fluorescence intensity analysis showed that the distance between IBPs on the ice surface is 7-20 nm (the ice-binding site of the protein is 3 nm long), and a correlation between the surface density to the measured TH activity was obtained. Microfluidic solution exchange experiments show that both moderate and hyperactive IBPs prevent the growth of ice crystals after the removal of the protein solution. These results have a significant contribution to understanding the IBP mechanism and can be helpful in applying these proteins in different fields.

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1) Celik, Y., Drori, R., et al., Proc Natl Acad Sci U S A, 2013. 110(4): p. 1309-14.

3323-Pos Board B51

Can a Protein's Evolutionary Fate be Predicted from its Structure?

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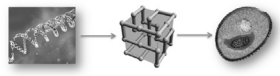
A fundamental question in evolution is the role of a protein's 3D structure in determining its evolvability. We observe that different protein structures vary widely in their ability to respond adaptively to simulated selective pressures, even with the aid of folding chaperones. This variation that cannot be explained by factors such as stability, that are already understood to promote evolvability. In these simulations, lattice proteins evolve in a crowded, cell-like environment with complex dynamics between protein structure, protein-protein interactions, and cellular fitness, while remaining exactly solvable. We develop new structural metrics predictive of a protein's evolvability and corroborate the conclusions with analysis of protein folds in nature.



3324-Pos Board B52

Probing an Ancient Protein's Dynamics with NMR

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The tyrosine kinases Abl and Src are very similar in function and fold. However, the cancer drug imatinib exhibits highly selective binding for Abl compared to Src. For a long time it was assumed that these differences arise through a conformational selection mechanism of a DFG-loop, which allows binding of imatinib only if it is in the correct conformation. Recently, an additional induced fit step in the process of imatinib binding has been shown to account for the differences in binding of the drug to Abl in comparison with Src. To investigate the binding mechanism of imatinib to Abl we used resurrected enzymes along the nodes in a phylogenetic tree between the common ancestor of Abl and Src and the contemporary Abl kinase. We show that the binding affinities of each of the four resurrected ancestor enzymes are gradually increasing from the common ancestor towards the contemporary Abl and that the mechanism of binding is comprised of a conformational selection step followed by an induced fit step. To probe the conformational changes the enzyme undergoes upon imatinib binding, we use CPMG relaxation dispersion experiments. We show that most of the exchanging residues are in an intermediate or fast time regime. Combining the CPMG data with molecular dynamics will allows us to get a more detailed look at which residues play an import role for the selectivity of imatinib towards Abl kinase.

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Exploring the Energy Landscape through Ancestral Proteins

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How does a protein's energy landscape change over an evolutionary time scale, and could a study of ancestral sequences reveal novel features of the landscape? Although there has been significant advances protein structure prediction from its primary sequence, extracting features of the entire energy landscape remains a challenge. There is limited understanding on how variations in the sequence that take place over the course of an evolutionary time scale change the landscape to yield novel properties and function. Past research on the landscape has focused on extant proteins, which represent only a small portion of proteins that have existed since the origin of life. Examining the ancestors of current proteins should provide additional insight into the properties of proteins and the process of protein evolution, and ultimately, further our understanding of the depth of information encoded within an amino acid sequence.

We conducted an ancestral sequence reconstruction of ribonuclease H1, a biophysically well-characterized protein. A total of seven ancestral proteins along the mesophilic and thermophilic lineages were constructed and studied. This study reports on the characterization of the folding pathway and stability of the last common ancestral protein between E. coli and T. thermophilus ribonucleases H.

3326-Pos Board B54

The Effective Temperature of Mutations: A General Mechanism for the Congruent Evolution of Robustness

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Genetic robustness is critical to the understanding of evolution, as phenotypically expressed variation is the fuel of natural selection. The origin of robustness, whether it evolves directly by natural selection or it is a correlated byproduct of other phenotypic traits, is unresolved. Examining microRNA (miRNA) genes of several eukaryotic species, Borenstein and Ruppin [1] showed that the structure of miRNA precursor stem-loops exhibits significantly increased mutational robustness in comparison with random RNA sequences with the same stem-loop structure. Introducing a novel measure of robustness based on the equilibrium thermodynamic ensemble of secondary structures of miRNA precursor sequences, we demonstrated [2] that, (i) miRNA are significantly more tolerant with respect to thermal and mutational perturbations (exhibit enhanced thermodynamic and mutational robustness) than samples of random RNA sequences with the same structure, (ii) the biophysics of RNA folding induces a high level of correlation between the responses to mutational and thermodynamic perturbations.

Motivated by the striking similarity between the effects of mutational and thermodynamic perturbations that the observed high level of correlation implies, here we attempt to quantify this similarity. Extending our study to lattice proteins we demonstrate that the effects of mutations can very generally and with unanticipated precision be described as an effective temperature increase of the thermodynamic ensemble of structures [3]. This result presents a general mechanism for the congruent evolution of thermodynamic and mutational robustness in the context of molecular phenotypes, and provides an explanation of how increased stability can facilitate opportunities for molecular innovation by facilitating increased neutral variation.

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[2] Szollosi & Derenyi (2009) Congruent evolution of genetic and environmental robustness in microRNA. Mol. Biol. Evol.
[3] Szollosi & Derenyi (in preperation) The effective temperature of mutations and the congruent evolution of robustness.